



## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

<b>(51) International Patent Classification <sup>7</sup> :</b> <b>C12N 15/52, 15/53, 15/82, 5/10, A01H 5/00</b>	<b>A1</b>	<b>(11) International Publication Number:</b> <b>WO 00/53768</b> <b>(43) International Publication Date:</b> 14 September 2000 (14.09.00)
<b>(21) International Application Number:</b> PCT/EP00/01850 <b>(22) International Filing Date:</b> 3 March 2000 (03.03.00)  <b>(30) Priority Data:</b> 199 09 637.6 5 March 1999 (05.03.99) DE  <b>(71) Applicant (for all designated States except US):</b> GREENOVATION PFLANZENBIOTECHNOLOGIE GMBH [DE/DE]; Sonnenstrasse 5, D-79104 Freiburg im Breisgau (DE).  <b>(72) Inventors; and</b> <b>(75) Inventors/Applicants (for US only):</b> BEYER, Peter [DE/DE]; In der Etzmatt 10, D-79423 Heitersheim (DE). POTRYKUS, Ingo [DE/CH]; Im Stigler 54, CH-4312 Magden (CH).  <b>(74) Agent:</b> JOACHIM STÜRKEN PATENTANWALTSGESELLSCHAFT MBH; Engesserstrasse 4b, D-79108 Freiburg im Breisgau (DE).	<b>(81) Designated States:</b> AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).  <b>Published</b> <i>With international search report.</i> <i>Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>	
<b>(54) Title:</b> METHOD FOR IMPROVING THE AGRONOMIC AND NUTRITIONAL VALUE OF PLANTS  <div data-bbox="544 1039 1055 1480"><pre>graph TD; IPP[IPP/DMAPP] --&gt; GGPP[GGPP]; GGPP --&gt; Tocopherols; GGPP --&gt; Gibberellins; GGPP --&gt; Chlorophylls; GGPP --&gt; Plastiquinone; GGPP --&gt; Phytoquinone; GGPP --&gt; Phytoene; Phytoene --&gt; Carotenoids</pre></div>		
<b>(57) Abstract</b> <p>The present invention provides means and methods of transforming plant cells, seeds, tissues or whole plants in order to yield transformants capable of expressing all enzymes of the carotenoid biosynthesis pathway that are essential for the targeted host plant to accumulate carotenes and/or xanthophylls of interest. The present invention also provides DNA molecules designed to be suitable for carrying out the method of the invention, and plasmids or vector systems comprising said molecules. Furthermore, the present invention provides transgenic plant cells, seeds, tissues and whole plants that display an improved nutritional quality and contain such DNA molecules and/or that have been generated by use of the methods of the present invention.</p>		

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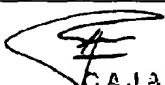
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A. The indications made below relate to the microorganism referred to in the description on page <u>20</u> , line <u>32</u>	
B. IDENTIFICATION OF DEPOSIT Further deposits are identified on an additional sheet <input checked="" type="checkbox"/>	
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Address of depositary institution (including postal code and country) <b>Mascheroder Weg 1b 38124 Braunschweig Germany</b>	
Date of deposit <b>March 5, 1999</b>	Accession Number <b>DSM 1271<sup>4</sup><sub>3</sub></b>
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Claims:

1. An isolated DNA molecule comprising a nucleotide sequence providing one or more expression cassettes capable of directing production of one or more enzymes specific for the carotenoid biosynthesis pathway selected from the group consisting of:
  - phytoene synthase derived from plants, fungi or bacteria,
  - phytoene desaturase derived from plants, fungi or bacteria,
  - $\zeta$ -carotene desaturase derived from plants, and
  - lycopene cyclase derived from plants, fungi or bacteria,under the proviso that an expression cassette capable of directing production of phytoene synthase alone is excluded.
2. The DNA molecule according to claim 1, wherein said expression cassette comprises one or more genes or cDNAs coding for plant, fungi or bacterial phytoene synthase, plant, fungi or bacterial phytoene desaturase, plant  $\zeta$ -carotene desaturase, or plant, fungi or bacterial lycopene cyclase, each operably linked to a suitable constitutive, inducible or tissue-specific promoter allowing its expression in plant cells, seeds, tissues or whole plants, under the proviso that an expression cassette comprising a gene or cDNA coding for phytoene synthase alone is excluded.
3. The DNA molecule according to claim 1 or 2, further comprising at least one selectable marker gene or cDNA operably linked to a constitutive, inducible or tissue-specific promoter sequence allowing its expression in plant cells, seeds, tissues or whole plants.
4. The DNA molecule according to any of claims 1 to 3, wherein the nucleotide sequence coding for phytoene synthase originates from plants, preferably expressed under the control of a tissue-specific promoter.
5. The DNA molecule according to any of claims 1 to 4, wherein the nucleotide sequence coding for phytoene desaturase originates from bacteria and is fused with a suitable plastid transit peptide encoding sequence, both of which preferably are expressed under the control of a tissue-specific or constitutive promoter.

6. The DNA molecule according to any of claims 1 to 5, wherein the nucleotide sequence coding for lycopene cyclase originates from plants, preferably expressed under the control of a tissue-specific or constitutive promoter.
- 5 7. The DNA molecule according to any of claims 2 to 6, wherein the selectable marker gene or cDNA is hygromycin phosphotransferase under the control of a constitutive promoter.
8. The DNA molecule according to any of claims 1 to 7, wherein said nucleotide sequence comprises functional expression cassettes for both phytoene synthase and bacterial or  
10 fungi phytoene desaturase.
9. The DNA molecule according to any of claims 1 to 7, wherein said nucleotide sequence comprises a functional expression cassette for lycopene cyclase.
- 15 10. The DNA molecule according to claim 5 or 8, wherein said plastid transit peptide sequence is derived from the pea Rubisco small subunit (tp).
11. A plasmid or vector system comprising one or more DNA molecules according to any of claims 1 to 10.
- 20 12. A plasmid or vector system according to claim 11, which is derived from *Agrobacterium tumefaciens*.
13. A transgenic plant cell, seed, tissue or whole plant that contains a DNA molecule  
25 according to any of claims 1 to 10.
14. A transgenic plant cell, seed, tissue or whole plant according to claim 13, selected from the group consisting of eukaryotic alga, embryophytes comprising *Bryophyta*, *Pteridophyta* and *Spermatophyta* such as *Gymnospermae* and *Angiospermae*, the latter  
30 including *Magnoliopsida*, *Rosopsida*, and *Liliopsida* ("monocots").

15. A transgenic plant cell, seed, tissue or whole plant according to claim 14, selected from the group consisting of grain seeds, with rice, wheat, barley, oats, amaranth, flax, triticale, rye, and corn being preferred; oil seeds, with *Brassica* seeds, cotton seeds, soybean, safflower, sunflower, coconut, and palm being preferred; other edible seeds or seeds with edible parts selected from the group consisting of pumpkin, squash, sesame, poppy, grape, mung beans, peanut, peas, beans, radish, alfalfa, cocoa, coffee, hemp; tree nuts, with walnuts, almonds, pecans, and chick-peas being preferred; potatoes, carrots, sweet potatoes, tomato, pepper, cassava, willows, oaks, elm, maples, apples, bananas; and ornamental flowers, with lilies, orchids, sedges, roses, buttercups, petunias, phlox, violets, and sunflowers being preferred.
16. A method of transforming plant cells, seeds, tissues or whole plants in order to yield transformants capable of expressing all enzymes of the carotenoid biosynthesis pathway necessary to produce carotenes and xanthophylls of interest, comprising the transformation of said plant cells, seeds, tissues or whole plants with one or more DNA molecules according to any of claims 1 to 10, or with a plasmid or vector system according to claim 11 or 12.
17. A method according to claim 16, wherein said host plant cells, seeds or tissues selected for transformation normally are carotenoid-free.
18. A method according to claim 16, wherein said host plant cells, seeds or tissues selected for transformation normally contain carotenoids in amounts desired to enlarge or modify.
19. A transformed whole plant regenerated from transformants yielded according to any of claims 16 to 18, or parts thereof, selected from the group consisting of eukaryotic alga, embryophytes comprising *Bryophyta*, *Pteridophyta* and *Spermatophyta* such as *Gymnospermae* and *Angiospermae*, the latter including *Magnoliopsida*, *Rosopsida*, and *Liliopsida* ("monocots").

20. A transformed whole plant or part thereof according to claim 19, selected from the group consisting of grain seeds, with rice, wheat, barley, oats, amaranth, flax, triticale, rye, and corn being preferred; oil seeds, with *Brassica* seeds, cotton seeds, soybean, safflower, sunflower, coconut, and palm being preferred; other edible seeds or seeds with edible parts selected from the group consisting of pumpkin, squash, sesame, poppy, grape, mung beans, peanut, peas, beans, radish, alfalfa, cocoa, coffee, hemp; tree nuts, with walnuts, almonds, pecans, and chick-peas being preferred; potatoes, carrots, sweet potatoes, tomato, pepper, cassava, willows, oaks, elm, maples, apples, bananas; and ornamental flowers, with lilies, orchids, sedges, roses, buttercups, petunias, phlox, violets, and sunflowers being preferred.



# INTERNATIONAL SEARCH REPORT

Inter. Application No.  
PCT/EU/01850

<b>A. CLASSIFICATION OF SUBJECT MATTER</b> IPC 7 C12N15/52 C12N15/53 C12N15/82 C12N5/10 A01H5/00		
According to International Patent Classification (IPC) or to both national classification and IPC		
<b>B. FIELDS SEARCHED</b> Minimum documentation searched (classification system followed by classification symbols) IPC 7 C12N A01H		
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched		
Electronic data base consulted during the international search (name of data base and, where practical, search terms used) EPO-Internal, BIOSIS, CHEM ABS Data		
<b>C. DOCUMENTS CONSIDERED TO BE RELEVANT</b>		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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X	BARTLEY GLENN E ET AL: "Two Arabidopsis thaliana carotene desaturases, phytoene desaturase and zeta-carotene desaturase, expressed in Escherichia coli, catalyze a poly-cis pathway to yield pro-lycopene." EUROPEAN JOURNAL OF BIOCHEMISTRY, vol. 259, no. 1-2, January 1999 (1999-01), pages 396-403, XP000925505 ISSN: 0014-2956 the whole document	1,11
<div style="display: flex; justify-content: space-around;"> <span>—</span> <span>—/—</span> </div>		
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Date of the actual completion of the international search  <div style="text-align: center; font-weight: bold;">24 July 2000</div>		Date of mailing of the international search report  <div style="text-align: center; font-weight: bold;">07/08/2000</div>
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Int. Application No.  
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Inter. Application No  
PCT/EP 00/01850

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

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Information on patent family members

Inter Publication No

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